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(54) Title: 1,3-DIPOLAR CYCLOADDITIONS TO POLYPYRROLIC MACROCYCLES			
(57) Abstract  Methods of modifying polypyrrolic macrocycles by use of a 1,3-dipolar cycloaddition is described. The methods may be used to produce compounds for further derivatization to produce photosensitizing agents of interest.			

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## 1,3-DIPOLAR CYCLOADDITIONS TO POLYPYRROLIC MACROCYCLES

### RELATED APPLICATIONS

This application claims benefit of priority from U.S. Provisional Application  
5 60/129,324, filed April 14, 1999, which is hereby incorporated by reference as if fully  
set forth.

### FIELD OF THE INVENTION

The field of invention is the design and synthesis of compounds, useful in  
10 photodynamic therapy and related applications of photoactive compound technology.  
In particular, the present invention relates to methods to modify polypyrrolic  
macrocycle compounds, such as porphyrins, via a 1,3-dipolar cycloaddition reaction  
to produce intermediates that may be further derivatized to produce unique  
polypyrrolic macrocycle derivatives. In particular, the invention relates to the use of  
15 the carbonyl ylide class of 1,3-dipoles to modify polypyrrolic macrocycle compounds  
and produce intermediates for further derivatization by conventional chemical  
reactions. The invention also relates to the resulting compounds as members of this  
class. The resultant polypyrrolic macrocycle derivatives produced via such  
intermediates are useful as:

- 20 - photosensitizers for photodynamic therapy;
- chelators for radionuclides;
- MRI contrast agents (i.e., chelators for paramagnetic metals);
- other biomedical uses; and
- 25 - technical uses for infrared absorbing dyes, such as imaging, data recording  
and printing.

### BACKGROUND OF THE INVENTION

Photodynamic therapy (PDT) generally involves the administration of  
compounds that are capable of absorbing light, typically in the visible range, but also  
30 in the near ultraviolet, followed by irradiation of locations in the subject for which a  
toxic, modifying or inhibitory effect is desired. PDT was initially developed using  
hematoporphyrin and related compounds in the treatment of tumors, as it appeared

The methods and compounds of the invention provide particularly useful new additions to the repertoire of compounds useful in photodynamic therapy (PDT) as well as means to expand this repertoire in a methodical fashion. The methods of the invention take advantage of the ability to modify polypyrrolic macrocycles by the use of a 1,3-dipolar cycloaddition reaction (see Huisgen, R., *Angew. Chem.* 1963, 75, 604; Huisgen, R., *Angew. Chem.* 1968, 80, 329; and Huisgen, R., *Proc. Chem. Soc.* 1961, 357). These types of reactions have become prominent in organic chemistry due to the vast number of bonds that undergo transformations (see Bianchi, G.; et al. *In The Chemistry of Functional Groups A*; Patai, S. Ed., Interscience: New York, 1977, 369; Stuckwisch, C.G., *Synthesis* 1973, 469; and Kaufmann, T., *Angew Chem. Int. Ed. Engl.* 1974, 13, 627). A "1,3-dipole" is a species that is represented by a zwitterionic octet structure and undergoes 1,3-cycloadditions with a multiple-bond system, the "dipolarophile." The 1,3-dipolar cycloaddition is a  $[3 + 2 \rightarrow 5]$  cycloaddition which normally forms a five-membered heterocyclic ring. The ring closure is effected by cyclic electron shifts which form two new  $\sigma$  bonds at the expense of  $\pi$  bonds. Over 18 different types of 1,3-dipoles have been employed in such reactions, presenting numerous possibilities for variation over and above the variety due to the wide diversity of the nature of the dienophile.

All 1,3-dipoles incorporate an onium center whose positive charge neutralizes the negative charge on one of the terminal atoms to form a heteroallyl anion which bears no net charge. Two of the four allylic  $\pi$  electrons can be delocalized at the center atom. The terminal centers of the dipoles can be either nucleophilic or electrophilic - the key to the reactivity of all 1,3-dipoles.

Formally, there are two types of 1,3-dipoles: those in which the central atom is sp-hybridized and those whose central atom is sp<sup>2</sup>-hybridized. The later group have allyl anion type  $\pi$  systems with four electrons in three parallel atomic  $\pi$  orbitals perpendicular to the plane of the dipole. This type of 1,3-dipole is bent and the central atom can be oxygen, nitrogen or sulfur. 1,3-Dipoles having an sp-hybridized central atom are referred to as propargyl or allenyl types. These are linear and the central atom is confined to nitrogen.

macrocycle. M is a metal selected from the group consisting of Ni(II), Cu(II), Zn(II), Fe(III)Cl, Sn, Ge, Si, Ga, Al, Mn(III), Gd(III), In and Tc;

R<sub>1</sub> through R<sub>6</sub> are independently a hydrogen atom, a lower alkyl group, a lower alkyl carboxylic acid or acid ester group, keto, hydroxy, nitro, amino or a group that, taken together with another ring, ring substituent or meso-substituent, forms a fused 5- or 6-membered ring.

Similarly, R<sub>x</sub> and R<sub>y</sub> are substituents formed from further derivatization of moieties introduced upon 1,3-dipolar cycloaddition to the macrocycle. Preferably, the cycloaddition results in a macrocycle intermediate containing one or more cyano groups. Upon further derivatization, R<sub>x</sub> and R<sub>y</sub> are independently selected from the group consisting of a hydrogen atom, a lower alkyl group, a lower alkyl carboxylic acid or acid ester group, keto, hydroxy, nitro, amino or a group that, taken together with another ring, ring substituent or meso-substituent, forms a fused 5- or 6-membered ring.

With respect to all R<sub>n</sub> groups, the other group covalently bonded to the carbon atom to which the R<sub>n</sub> is attached may be independently a hydrogen atom or a hydroxyl group.

Ph<sup>1</sup>, Ph<sup>2</sup>, Ph<sup>3</sup> and Ph<sup>4</sup> independently represent a group selected from H, substituted or unsubstituted alkyl groups, or substituted or unsubstituted aromatic rings, which may be the same or different.

Also included within the scope of the formulas are the salts and the metallated and/or labeled and/or conjugated forms thereof.

The invention is also directed to methods to make the compounds of the formula by modification of a base polypyrrolic macrocycle with a carbonyl ylide to form an intermediate which may then be subsequently isomerized, reduced, and/or derivatized.

Also included in the invention as a preferred embodiment are the intermediates and derivative compounds involved in the production of the formula and pharmaceutical compositions containing these compounds as well a methods to perform PDT using them.

the introduction of a carbon atom via production of a primary amine. This carbon atom introduction may be used to lengthen a carbon chain by one atom.

The ability to produce an amine also makes possible the further derivatization of the intermediate with the vast number of amine based reactions, including  
5 alkylation, amide formation, derivatization to a diazonium salt for subsequent replacement (e.g. by -Cl, -Br, -I, -CN, and -OH) or coupling, and imine formation.

Alternatively, the cyano containing moiety introduced into a polypyrrolic macrocycle may be base hydrolyzed to permit further derivatization. Such hydrolysis, for example, may be used to generate an acid and/or a carboxylate moiety that may be  
10 retained or further derivatized. Known chemistries of acids and carboxylates may be used in the production of other moieties, including  $\alpha$ -alkylations, esters, amides, and thioamides.

The terms "intermediate" or "intermediate compound" are used to describe any compound of the invention that may be further derivatized to another compound.  
15 Such "intermediates" may be stable or unstable compounds under normal conditions and may or may not be photoactivatable. The terms also include compounds that may be suitable for use as photosensitizing agents with or without further derivatization.

In a preferred embodiment of the invention, the carbonyl ylide used is tetracyanoethylene oxide (TCNEO), which has been used with olefins, acetylenes and  
20 benzene at high temperatures (see Linn, W.J., et al. J. Am. Chem. Soc. 1965, 87, 3651; Lown, J.W., et al. Can. J. Chem. 1972, 50, 534; Linn, W.J., et al. J. Org. Chem. 1969, 34, 2146; Linn, W.J. et al. J. Am. Chem. Soc. 1965, 87, 3657; and Linn, W.J., et al. J. Am. Chem. Soc. 1965, 87, 3665). The products were those formed from [3+  
2] cycloadditions with the carbonyl ylide. A first-order electrocyclic ring opening via  
25 cleavage of the carbon-carbon bond of the epoxide to the 1,3-dipole occurs (see Linn above). See Figure 2. The TCNEO dipole adds cleanly to a variety of olefins, including aromatic substrates. Aromatic dipolarophiles include benzene, naphthalene, toluene, 1,3-cyclohexadiene and furan. These reagents produced the corresponding 1:1 adducts in 18-73% yields (Linn, W.J. et al. J. Am. Chem. Soc. 1965, 87, 3657).

30 For example, naphthalene reacts with one equivalent of TCNEO in 1,2-dibromoethane to give a single monoadduct at the 1,2-position in 73% yield after 4.75

TCNEO. Reaction of 1.5 eq. TCNEO with DPP after refluxing in toluene for 3 hours produced two compounds: a red nonpolar compound 1 and a purple compound 2.

See Figure 5.

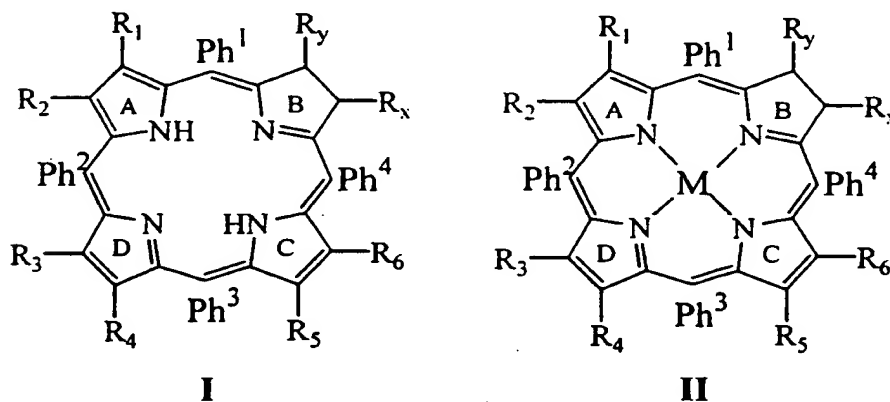
The absorbance spectrum of compound 1 product was very unusual with a  
5 split Soret at 376 and 416 nm and two intense overlapping Q bands at 510 and 534 nm. The proton NMR spectrum of this compound was quite different from that of the starting material. Two doublets at 6.5 ppm and 6.6 ppm, both integrating for 2 protons each, and a triplet at 7.1 ppm which integrated for 4 protons hinted that the molecule had lost its aromaticity but retained some sort of conjugation. Additionally,  
10 there were no peaks below 0 ppm to represent the NH protons. Instead there was a sharp singlet peak at 13.9 ppm which integrated for two protons. Protons on the nitrogen atoms of dipyrromethenes have previously been observed in this range. The protons of the two phenyl groups were observed at 7.4-7.5 as a large multiplet. Mass spectrometry of the red compound 1 revealed a compound at m/e 540 and with a  
15 molecular formula of  $C_{35}H_{20}N_6O$ . This corresponded to DPP plus  $C_3ON_2$  and a loss of two protons.

The purple compound 2 also displayed an unusual absorbance spectrum with one broad Soret band at 400 nm and one intense band at 562 nm. The proton NMR spectrum of the compound had a doublet representing four protons at 6.65 ppm, a  
20 doublet at 7.2 ppm also representing four protons, a multiplet representing the phenyl protons at 7.4-7.55 ppm and a singlet at 13.75 ppm representing two protons. The peaks at 6.65 and 7.2 ppm (both doublets with  $J=4.5$  Hz) and the lack of splitting of the Soret and Q-band both indicated a higher degree of symmetry as compared to the  
red product compound 1. High resolution mass spectrometry determined the  
25 molecular formula of the parent ion at m/e 588 to correspond to a molecular formula of  $C_{38}H_{20}N_8$ . Without being bound by theory, the mechanism of formation of this compound presumably involves electrophilic aromatic substitution at the meso positions. A proposed scheme for the formation of compound 2 is shown in Figure 6. Compound 1 may be formed by hydrolysis of compound 2.

30 Both compounds (1 and 2) may be considered intermediates for the production of additional DPP derivatives since the cyano groups of compound 2 and the cyano

The ability to generate new reactive intermediates such as 5,10,15,20-tetraphenyl-2,3-(3'-dicyano)cyclopropano-2,3-chlorin and compounds 1, 2, 3, and 4 has important consequences. First, the intermediates offer new approaches to the synthesis of new photoactive agents. Second, the reaction offers a new means of generating known photosensitizers that may be more economical or practical than previously known methods.

The new photosensitizers made possible by the invention include those encompassed by the formulas



10

wherein formula I represents the structure of a polypyrrolic macrocycle of the invention and formula II represents the structure of the metallated form of the macrocycle. M is a metal selected from the group consisting of Ni(II), Cu(II), Zn(II), Fe(III)Cl, Sn, Ge, Si, Ga, Al, Mn(III), Gd(III), In and Tc. Preferably, the metal is one which is either not toxic to higher organisms or readily removed or exchanged in favor of another, less toxic metal. Of course toxic metals remain encompassed by the invention for use in circumstances where the toxicity may be advantageously used to suppress the growth or proliferation of organisms, such as microorganisms.

15

R<sub>1</sub> through R<sub>6</sub> can be any one of a large number of ring substituents, so long as they do not interfere with the cycloaddition reaction outlined above. Preferably, R<sub>1</sub> through R<sub>6</sub> are independently a hydrogen atom; a lower alkyl group, such as methyl, ethyl, n-propyl, isopropyl, t-butyl and n-pentyl; a lower alkyl carboxylic acid, such as formyl, carboxymethyl, carboxyethyl, carboxy-n-butyl, carboxy-sec-butyl, carboxy-n-

20

group such that formulas I and II are directed to monophenyl-, diphenyl-, triphenyl-, and tetraphenyl- substituted polypyrrolic macrocycles

When one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is an alkyl group, they preferably have from about 1 to about 18 carbon atoms, more preferably about 1 to 12 carbon atoms and, even more preferably, about 1-6 carbon atoms. Examples of typical alkyl groups are methyl, ethyl, isopropyl, sec-butyl, tert-butyl, n-pentyl and n-octyl.

When one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is an alkyl group, it may be unsubstituted or substituted with any group that does not interfere with the cycloaddition or reduction reactions. For example, when one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is an alkyl group may be substituted by a halogen atom, such as fluorine, chlorine or bromine; a hydroxy group, such as in pentoses and hexoses; thiol; or a carbonyl group, such as when the alkyl group is an aldehyde, ketone, carboxylic acid (e.g., a fatty acid) or ester or amide; a primary, secondary, tertiary, or quaternary amino group; nitrile; a phosphate group; a sulfonate group; and the like.

When one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is a cycloalkyl group, it preferably contains from about 3 to about 7 carbon atoms. Examples of typical cycloalkyl groups include cyclopropyl, cyclohexyl, and cycloheteroalkyl, such as glucopyranose or fructofuranose sugars. When one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is a cycloalkyl group, it may be unsubstituted or substituted with any group that does not interfere with the cycloaddition or reduction reactions. For example, when one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is a cycloalkyl group, they may be substituted by any of the same substituents described above for the case when one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is an alkyl group.

When one or more of  $\text{Ph}^1$ ,  $\text{Ph}^2$ ,  $\text{Ph}^3$  and  $\text{Ph}^4$  is an aryl group, it preferably contains from about 5 to about 12 carbon atoms, optionally containing one or more heteroatoms, and optionally including rings that are fused to the existing conjugated porphyrin ring structure. Examples of suitable aromatic rings include furan, thiophene, pyrrole, isopyrrole, 3-isopyrrole, pyrazole, 2-isimidazole, 1,2,3-triazole, 1,2,4-triazole, 1,2-dithiole, 1,3-dithiole, 1,2,3-oxathiole, isoxazole, oxazole, thiazole, isothiazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3,4-oxatriazole, 1,2,3,5-oxatriazole, 1,2,3-dioxazole, 1,2,4-dioxazole, 1,3,2-



- (1) having both (a) a highly polar, water-soluble region and (b) a highly hydrophobic, water-insoluble region; or
- (2) having both (a) a nonionic region and (b) an ionic region.

However, it should be noted that the invention also includes  $\beta,\beta'$ -dihydroxy meso-substituted chlorin, bacteriochlorin or isobacteriochlorin compounds having

5 substantially or exactly identical aryl substituents. Further, any aryl substituent chosen should also have no adverse effect on the ability of the compound to undergo the cycloaddition reaction.

- Preferably, X, X', Y, Y' and Z are independently (1) hydrogen; (2) halogen, such as fluoro, chloro, iodo and bromo; (3) lower alkyl, such as methyl, ethyl, n-propyl, isopropyl, t-butyl, n-pentyl and the like groups; (4) lower alkoxy, such as methoxy, ethoxy, isopropoxy, n-butoxy, t-pentoxy and the like; (5) hydroxy; (6) carboxylic acid or acid salt, such as  $-\text{CH}_2\text{COOH}$ ,  $-\text{CH}_2\text{COO}-\text{Na}^+$ ,  $-\text{CH}_2\text{CH}(\text{Br})\text{COOH}$ ,  $-\text{CH}_2\text{CH}(\text{CH}_3)\text{COOH}$ ,
- 10  $-\text{CH}(\text{Cl})-\text{CH}_2-\text{CH}(\text{CH}_3)-\text{COOH}$ ,  $-\text{CH}_2-\text{CH}_2-\text{C}(\text{CH}_3)_2-\text{COOH}$ ,  $-\text{CH}_2-\text{CH}_2-\text{C}(\text{CH}_3)_2-\text{COO}^-\text{K}^+$ ,  $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{COOH}$ ,  $\text{C}(\text{CH}_3)_3-\text{COOH}$ ,  $\text{CH}(\text{Cl})_2-\text{COOH}$  and the like; (7) carboxylic acid ester, such as  $-\text{CH}_2\text{CH}_2\text{COOCH}_3$ ,  $-\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_3$ ,  $-\text{CH}_2\text{CH}(\text{CH}_3)\text{COOCH}_2\text{CH}_3$ ,  $-\text{CH}_2\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{CH}_3$ ,  $-\text{CH}_2\text{CH}(\text{CH}_3)_2\text{COOCH}_2\text{CH}_3$ , and the like; (8)
- 20 sulfonic acid or acid salt, for example, group I and group II salts, ammonium salts, and organic cation salts such as alkyl and quaternary ammonium salts; (9) sulfonic acid ester, such as methyl sulfonate, ethyl sulfonate, cyclohexyl sulfonate and the like;
- (10) amino, such as unsubstituted primary amino, methylamino, ethylamino, n-propylamino, isopropylamino, 5-butylamino, sec-butylamino, dimethylamino,
- 25 trimethylamino, diethylamino, triethylamino, di-n-propylamino, methylethylamino, dimethyl-sec-butylamino, 2-aminoethanoxy, ethylenediamino, 2-(N-methylamino)heptyl, cyclohexylamino, benzylamino, phenylethylamino, anilino, N-methylanilino, N,N-dimethylanilino, N-methyl-N-ethylanilino, 3,5-dibromo-4-anilino, p-toluidino, diphenylamino, 4,4'-dinitrodiphenylamino and the like; (11) cyano; (12)
- 30 nitro; (13) a biologically active group; or (14) any other substituent that increases the amphiphilic nature of the compound of formula (I) or (II).

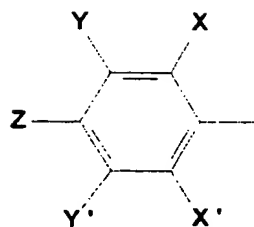
uridine; and 2'-deoxyribonucleosides, such as 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, and 2'-deoxythymidine.

Another category of biologically active groups that is particularly useful is any ligand that is specific for a particular biological receptor. The term "ligand specific for a receptor" refers to a moiety that binds a receptor at cell surfaces, and thus contains contours and charge patterns that are complementary to those of the biological receptor. The ligand is not the receptor itself, but a substance complementary to it. It is well understood that a wide variety of cell types have specific receptors designed to bind hormones, growth factors, or neurotransmitters. However, while these embodiments of ligands specific for receptors are known and understood, the phrase "ligand specific for a receptor", as used herein, refers to any substance, natural or synthetic, that binds specifically to a receptor.

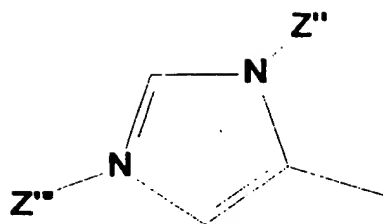
Examples of such ligands include: (1) the steroid hormones, such as progesterone, estrogens, androgens, and the adrenal cortical hormones; (2) growth factors, such as epidermal growth factor, nerve growth factor, fibroblast growth factor, and the like; (3) other protein hormones, such as human growth hormone, parathyroid hormone, and the like; and (4) neurotransmitters, such as acetylcholine, serotonin, dopamine, and the like. Any analog of these substances that also succeeds in binding to a biological receptor is also included.

Particularly useful examples of substituents tending to increase the amphiphilic nature of the compound of formula (I) include: (1) long chain alcohols, for example,  $-C_{12}H_{24}-OH$  where  $-C_{12}H_{24}$  is hydrophobic; (2) fatty acids and their salts, such as the sodium salt of the long-chain fatty acid oleic acid; (3) phosphoglycerides, such as phosphatidic acid, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, phosphatidyl glycerol, phosphatidyl 3'-O-alanyl glycerol, cardiolipin, or phosphatidal choline; (4) sphingolipids, such as sphingomyelin; and (5) glycolipids, such as glycosyldiacylglycerols, cerebroside, sulfate esters of cerebroside or gangliosides.

In a preferred embodiment, X, X', Y, Y' and Z are independently hydrogen, halogen, lower alkyl, lower alkoxy, hydroxy, carboxylic acid or acid salt, carboxylic acid ester, sulfonic acid or acid salt, sulfonic acid ester, substituted or unsubstituted



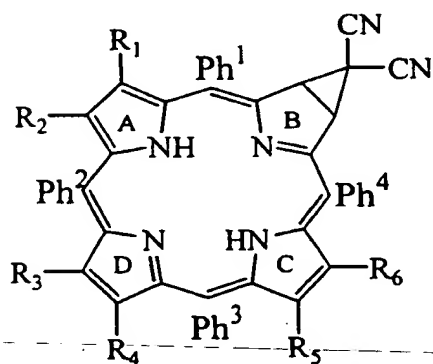
X	X'	Y	Y'	Z
-H	-H	-H	-H	-H
-OH	-H	-H	-H	-H
-H	-H	-OH	-H	-H
-H	-H	-H	-H	-OH
-H	-H	-OH	-OH	-OH
-H	-H	-H	-H	-SO <sub>3</sub> H(Na)
-CH <sub>3</sub>	-CH <sub>3</sub>	-H	-H	-CN
-H	-H	-OCH <sub>3</sub>	-OCH <sub>3</sub>	-OCH <sub>3</sub>
-H	-H	-H	-H	-COOH(Na)
-H	-H	-COOH(Na)	-COOH(Na)	-H
-H	-H	-H	-H	-C <sub>6</sub> H <sub>12</sub> COOH(Na)
-H	-H	-H	-C <sub>6</sub> H <sub>12</sub> COOH(Na)	-H
-H	-H	-C <sub>6</sub> H <sub>13</sub>	-H	-SO <sub>3</sub> H(Na)
-H	-H	-H	-COOH(Na)	-tert-Butyl
-H	-CH <sub>2</sub> NH <sub>2</sub>	-H	-H	-H
-H	-H	-H	-H	-NH <sub>2</sub>
-OH	-H	-H	-H	-CH <sub>2</sub> NH <sub>2</sub>
-H	-H	-H	-H	-C <sub>4</sub> H <sub>8</sub> NH <sub>2</sub>
-H	-H	-H	-COOCH <sub>3</sub>	-COOH(Na)
-OH	-H	-H	-COONHCH <sub>3</sub>	-H
-H	-H	-H	-COONHCH <sub>3</sub>	-COOH(Na)
-H	-H	-H	-imidazolyl	-H
-H	-H	-H	-glycinylyl	-H
-H	-H	-H	-steroidyl	-H
-H	-H	-H	-glycosyl	-H
-H	-H	-H	-H	-imidazolyl
-H	-H	-H	-H	-glycinylyl
-H	-H	-H	-H	-steroidyl
-H	-H	-H	-H	-glycosyl



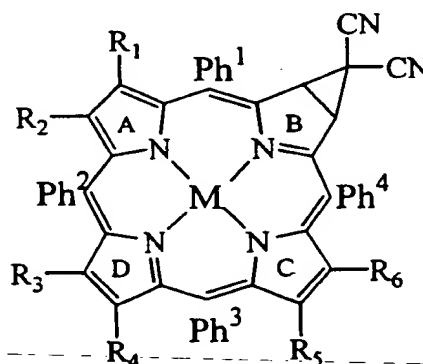
Z''	Z'''
-H	-H
-CH <sub>3</sub>	-H
-H	-CH <sub>3</sub>
-H	-C <sub>6</sub> H <sub>12</sub>
-C <sub>6</sub> H <sub>12</sub>	-H

The present invention also includes the intermediates produced by the cycloaddition reaction and represented by the formula

5



III



IV

wherein all variable positions are as defined above for formulas I and II.

In a particularly preferred aspect of the invention, derivatives are produced in such a manner that the polypyrrolic macrocyclic compound to be made will be an  
 10 amphiphilic molecule. By "amphiphilic" is meant that the molecule has become more asymmetric, such as

- (1) having both (a) a highly polar water-soluble region and (b) a highly hydrophobic, water-insoluble region;

inert atmosphere is usually provided by forming a protective blanket of an inert gas, such as argon, helium, or N<sub>2</sub>, over the reaction mixture or bubbling an inert gas through it. Other methods of providing an inert atmosphere include performing the reaction under reduced pressure to form an atmosphere of solvent vapor(s).

5           The reaction temperature in the reaction can vary widely depending on the reactivity of the reactants. However, the temperature should not be so high as to decompose the reactants or so low as to cause inhibition of the condensation or freezing of the solvent. In most cases, the reaction can take place at a temperature ranging from about room temperature, for reasons of convenience, to the reflux  
10           temperature of the reaction mixture, which typically varies from about 25 to about 150°C.

          The time required for the reaction will depend to a large extent on the temperature being used and the relative reactivities of the starting materials. Particularly when the meso-substituents are aryl, cycloalkyl, or a bulky alkyl group  
15           such as tert-butyl, the time required for the reaction may increase due to steric hindrance. Therefore, the reaction time can vary greatly, for example, from about five minutes to about two days. Typically, the time required may be from about 1 to about 24 hours, preferably from about 1 to about 18 hours, and more preferably from about 1 to about 2, about 3 or about 4 hours.

20           At the conclusion of the evaporation step (b), a residue remains, from which the meso-disubstituted tripyrrane can be isolated by any conventional means, such as by chromatography, crystallization, re-crystallization, sublimation, various combinations of these methods, and the like. Typically, two primary types of  
25           impurities must be removed from the residue: (1) high molecular weight polymeric materials; and (2) the dipyrromethane molecule corresponding to the desired tripyrrane product, which occurs as a by-product of the above-described reaction.

          The cyano containing intermediate compounds produced by the above methods may be reduced to produce amine groups for further derivatization of the compounds. In a preferred embodiment of the invention, lithium aluminum hydride is  
30           used for the reduction, but any suitable reducing agent known in the art may be used for reducing the compound.

to the substitution patterns, significantly improved biodistribution properties may be achieved by using the compounds of the invention.

The photosensitizers made from the compounds of the invention can be formulated into pharmaceutical compositions for administration to the subject or  
5 applied to an *in vitro* target using techniques generally known in the art. A summary of such pharmaceutical compositions may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA. The compounds of the invention can be used singly or as components of mixtures.

Generally, for the diagnosis or treatment of solid tumors, the compound of the  
10 invention, labeled or unlabeled, is administered systemically, such as by injection. Injection may be intravenous, subcutaneous, intramuscular, or even intraperitoneal. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in a liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline,  
15 dextrose, glycerol and the like. Of course, these compositions may also contain minor amounts of nontoxic, auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and so forth.

Systemic administration can be implemented through implantation of a slow release or sustained release system, by suppository, or, if properly formulated, orally.  
20 Formulations for these modes of administration are well known in the art, and a summary of such methods may be found, for example, in Remington's Pharmaceutical Sciences (*supra*).

----- If treatment is to be localized, such as for the treatment of superficial tumors  
or skin disorders, the compound can be administered topically using standard topical  
25 compositions, such as lotions, suspensions, or pastes.

The quantity of the photosensitizer compound to be administered depends upon the choice of active ingredient, the condition to be treated, the mode of administration, the individual subject, and the judgment of the practitioner. Depending on the specificity of the preparation, smaller or larger doses may be  
30 needed. For compositions that are highly specific to target tissues, such as those with a highly specific monoclonal immunoglobulin preparation or a specific receptor

**Example 2:** Preparation of compounds 1 and 2:

A solution of DPP (16 mg, 0.035 mmol) and tetracyanoethylene oxide (TCNEO) (7.5 mg, 0.05 mmol) in 1,2-dibromoethane (5 mL) was refluxed for 3 hours. The solvent was evaporated in vacuo, and preparative TLC performed (silica; 1:1 toluene:hexane) to yield compounds 1 and 2.

**Compound 1:**

RF 0.8 (silica -  $\text{CHCl}_3$ ); IR 2210(s), 1729.8 (m), 1571.7 (s), 1509 (m), 1384 (m), 1182 (s)  $\text{cm}^{-1}$   $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ) = 6.5 (d,  $J = 4.41$  Hz, 2H, H), 6.6 (d,  $J = 4.29$  Hz, 2H, H), 7.14 (t,  $J = 3.95$  Hz, 4H, H), 7.4-7.55 (m, 10H), 13.9 (s, 2H); UV-Vis ( $\text{CH}_2\text{Cl}_2$ ) max = 376, 416, 510, 534 nm; MS (EI)  $m/e$  calc'd for  $\text{C}_{35}\text{H}_{20}\text{ON}_6$ : 540.16986, found 540.17000 ( $\text{M}^+$ , 100%); yield 36%.

**Compound 2:**

RF 0.6 (silica -  $\text{CHCl}_3$ ); IR 2215 (s), 1650 (m), 1567 (m)  $\text{cm}^{-1}$   $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ) = 6.65 (d,  $J = 4.63$  Hz, 4H, H), 7.19 (d,  $J = 4.47$  Hz, 4H, H), 7.4-7.58 (m, 10H), 13.75 (s, 2H); UV-Vis ( $\text{CH}_2\text{Cl}_2$ ) max = 400, 562 nm; LRMS (EI)  $m/e$  588 ( $\text{M}^+$ , 100%), 563 ( $\text{M}^+ - \text{CN}$ , 40%); HRMS (EI)  $m/e$  calc'd for  $\text{C}_{38}\text{H}_{20}\text{N}_8$ : 588.18109, found 588.18193 ( $\text{M}^+$ , 100%); yield 45%.

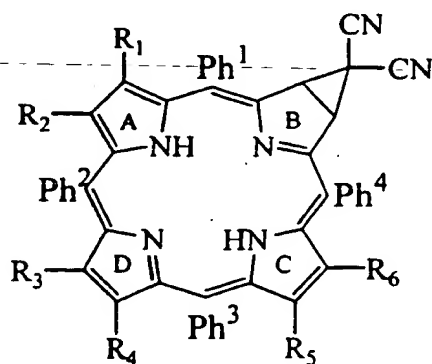
**Example 3:** Preparation of compound 3:

A solution of ZnDPP (50 mg, 0.07 mmol) and tetracyanoethylene oxide (TCNEO) (19 mg, 0.13 mmol) in 1,2-dibromoethane (5 mL) was refluxed for 2 hours. The solvent was evaporated in vacuo, and preparative TLC performed (silica; chloroform).

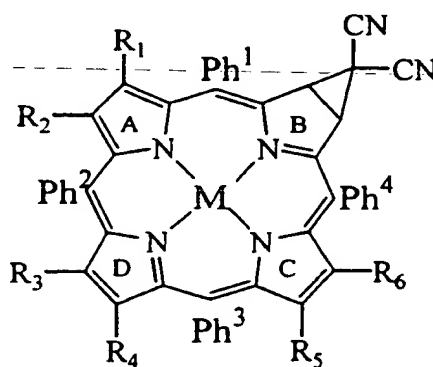
RF 0.6 (silica - 1% MeOH: $\text{CHCl}_3$ ); IR 2213.8(s), 1563.9 (m), 1492 (s)  $\text{cm}^{-1}$   $^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ) = 6.45 (d,  $J = 4.40$  Hz, 4H, H), 7.03 (d,  $J = 4.41$  Hz, 4H, H); UV-Vis ( $\text{CH}_2\text{Cl}_2$ ) max 420(sh), 458, 638 nm; LRMS (EI)  $m/e$  650 ( $\text{M}^+$ , 100%), 625 ( $\text{M}^+ - \text{CN}$ , 80%), 602 (60%); HRMS (EI)  $m/e$  calc'd for  $\text{C}_{38}\text{H}_{18}\text{N}_8\text{Zn}$ : 650.09460, found 650.09565 ( $\text{M}^+$ , 100%).

We claim:

1. A method of modifying a polypyrrolic macrocycle comprising reacting said macrocycle with a carbonyl ylide 1,3-dipole to produce an intermediate  
5 compound.
2. The method of claim 1 wherein said dipole is tetracyanoethylene oxide (TCNEO).
- 10 3. The method of claim 1 wherein said macrocycle is a photosensitizer.
4. The method of claim 3 wherein said photosensitizer is a porphyrin.
5. The method of claim 4 wherein said porphyrin is a  
15 tetraphenylporphyrin (TPP) or a diphenylporphyrin (DPP).
6. A compound produced by the method of any one of claims 1-4.
7. The compound of claim 6 having a structure represented by one of the  
20 following formulas



III



IV



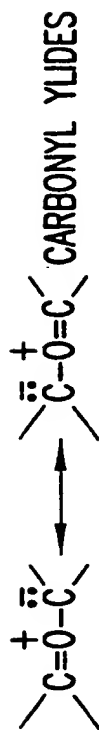


FIG. 1

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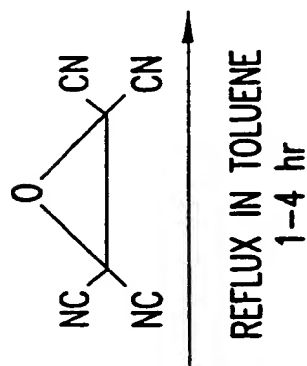
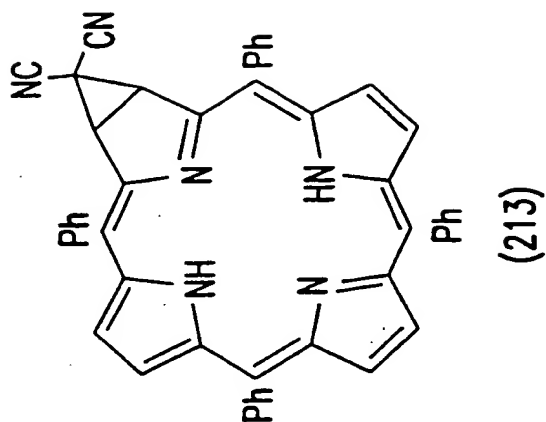
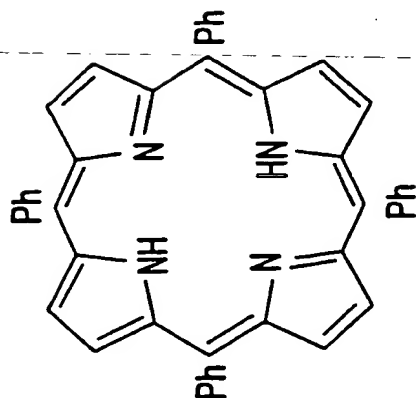


FIG.3



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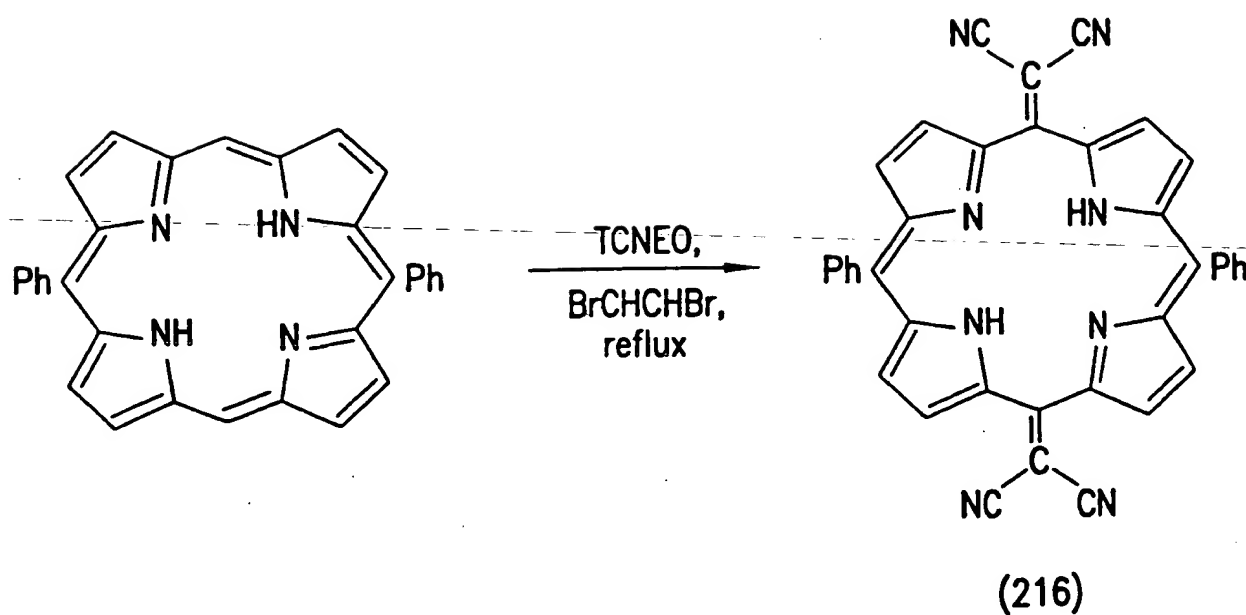
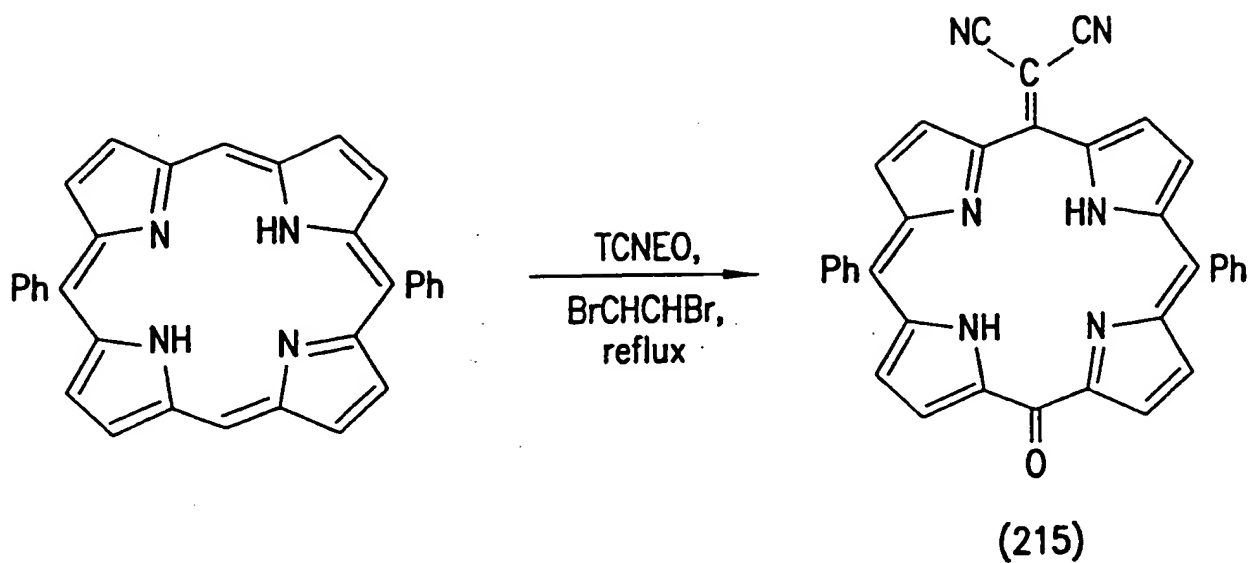


FIG.5

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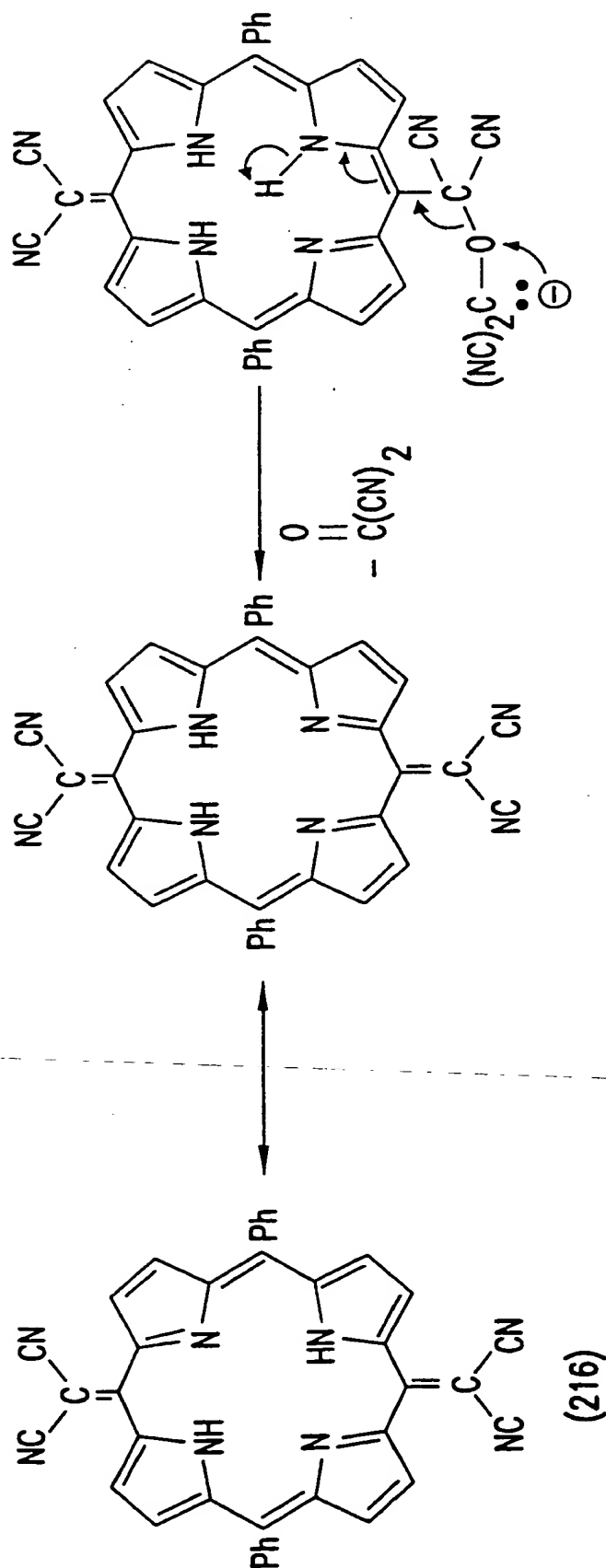


FIG. 6B

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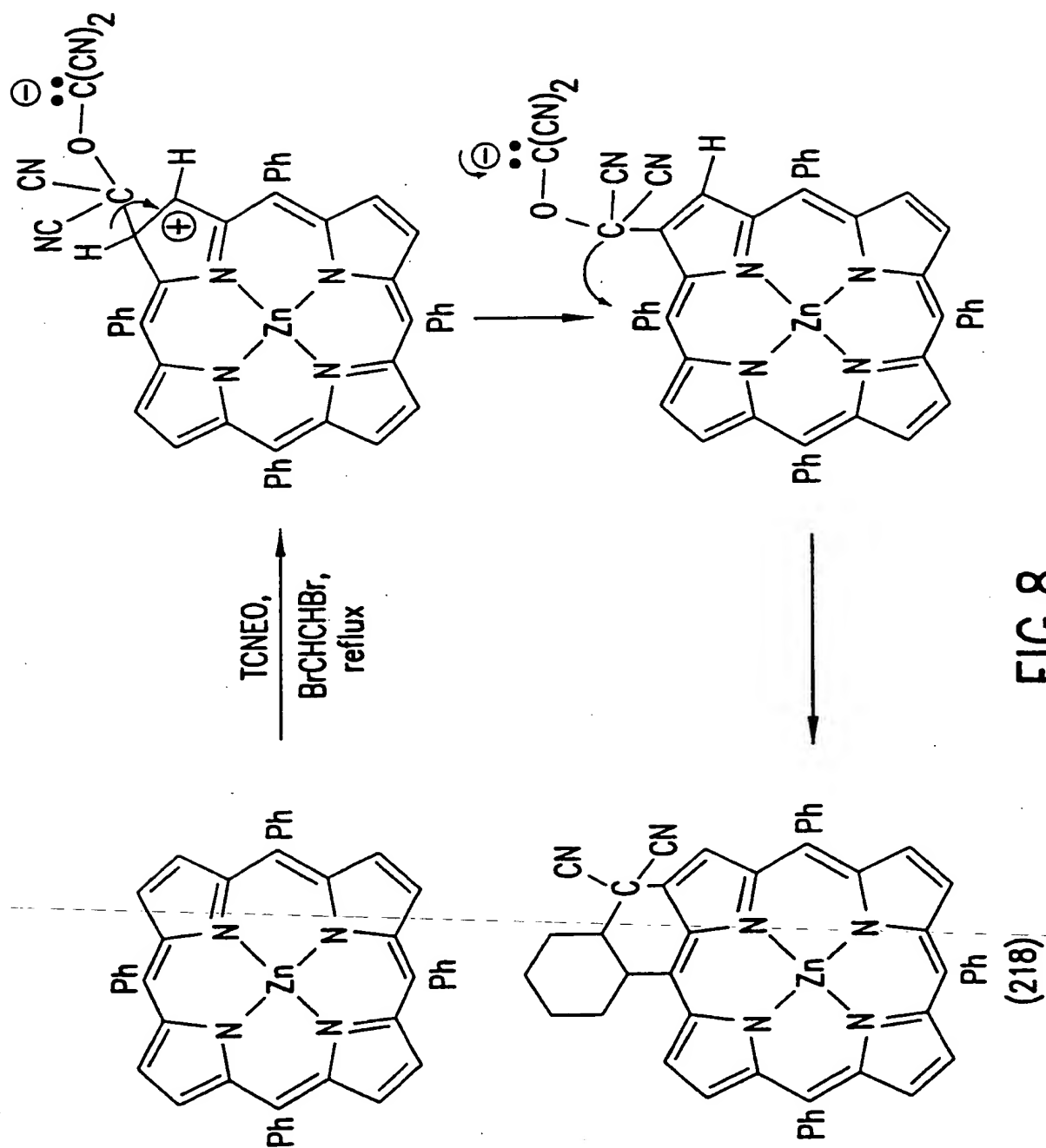


FIG. 8

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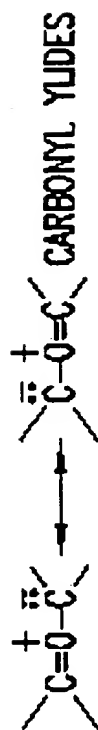
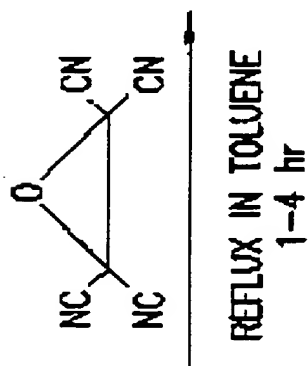
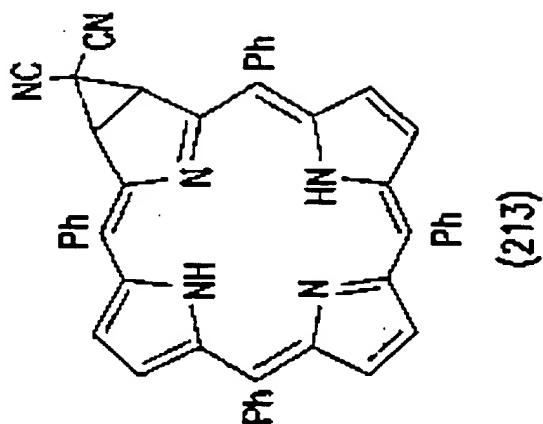
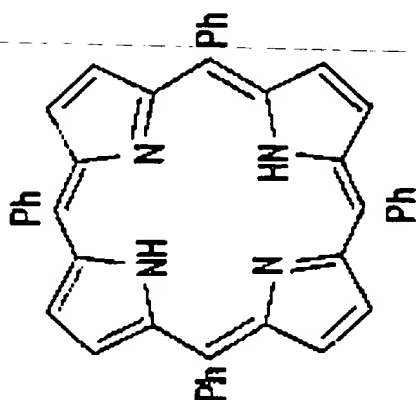


FIG. 1



REFLUX IN TOLUENE  
1-4 hr

FIG.3



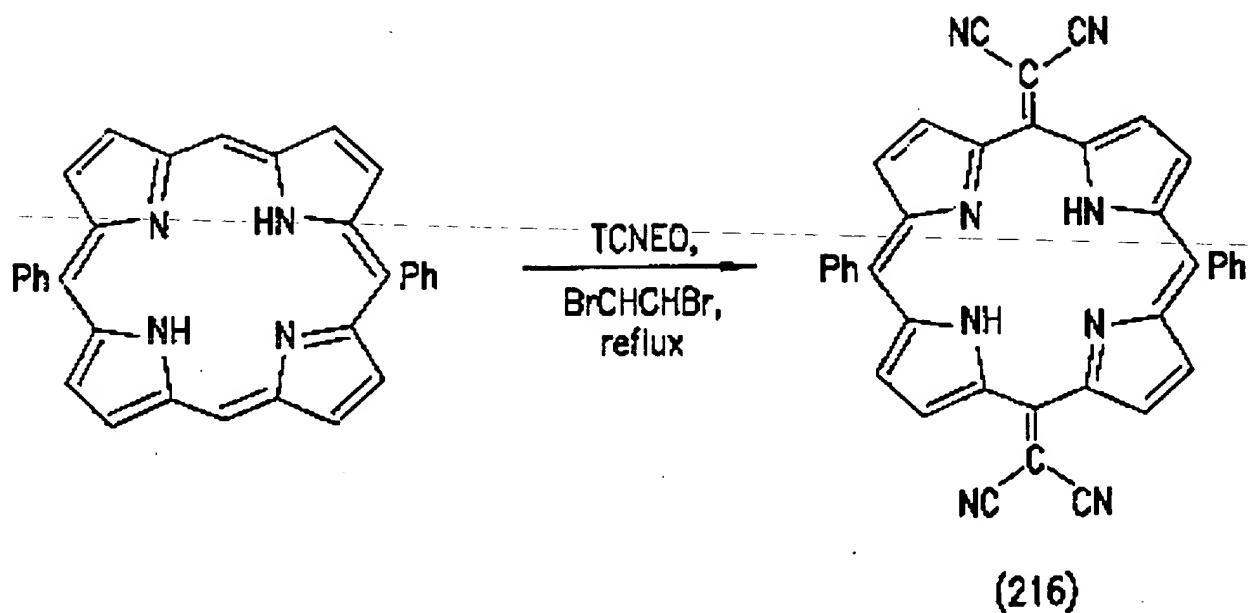
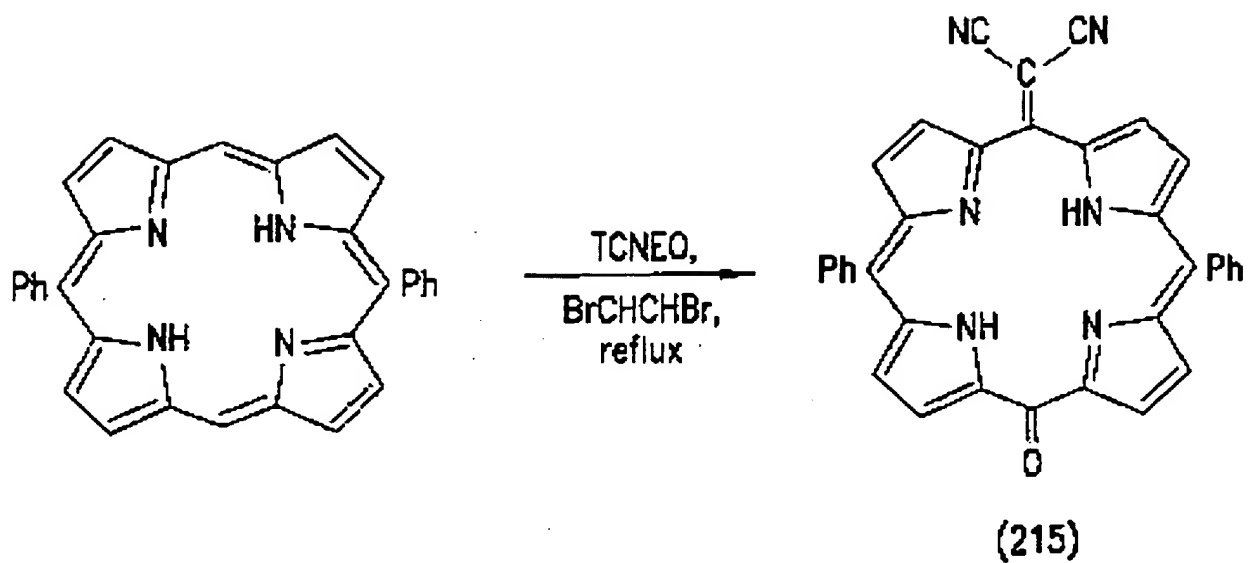


FIG.5



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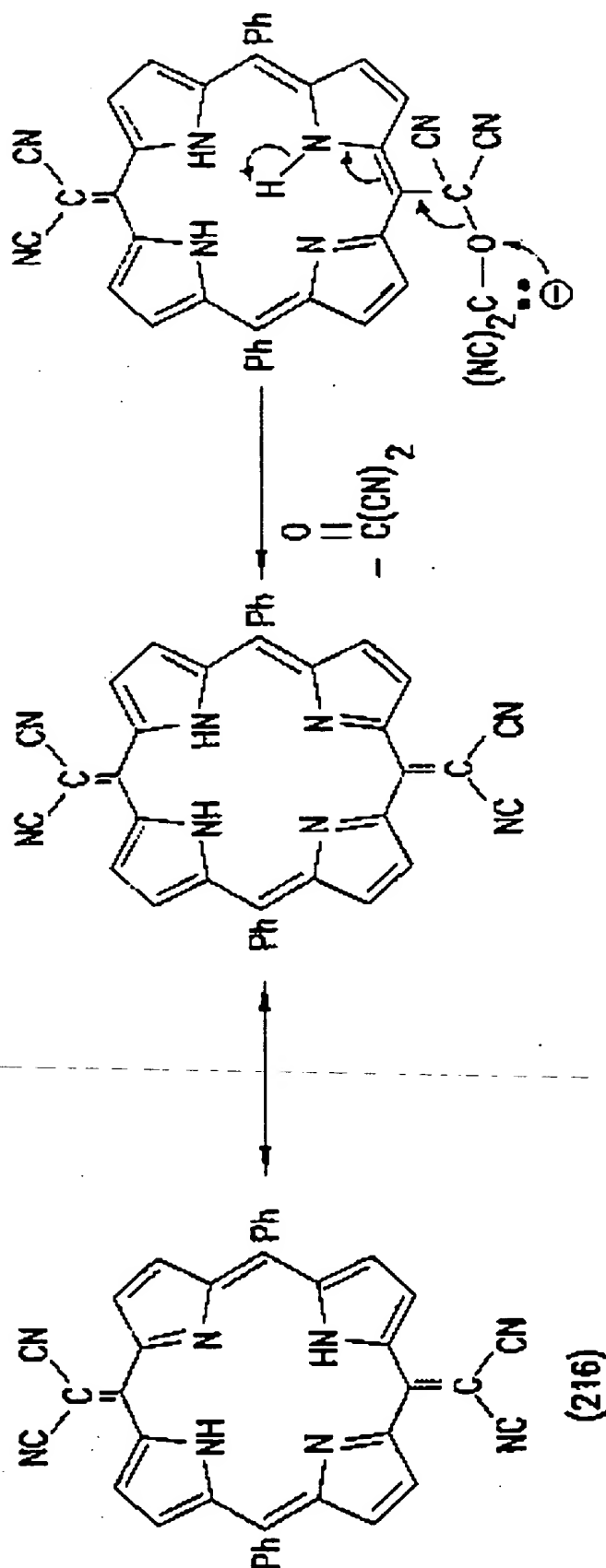


FIG. 6B

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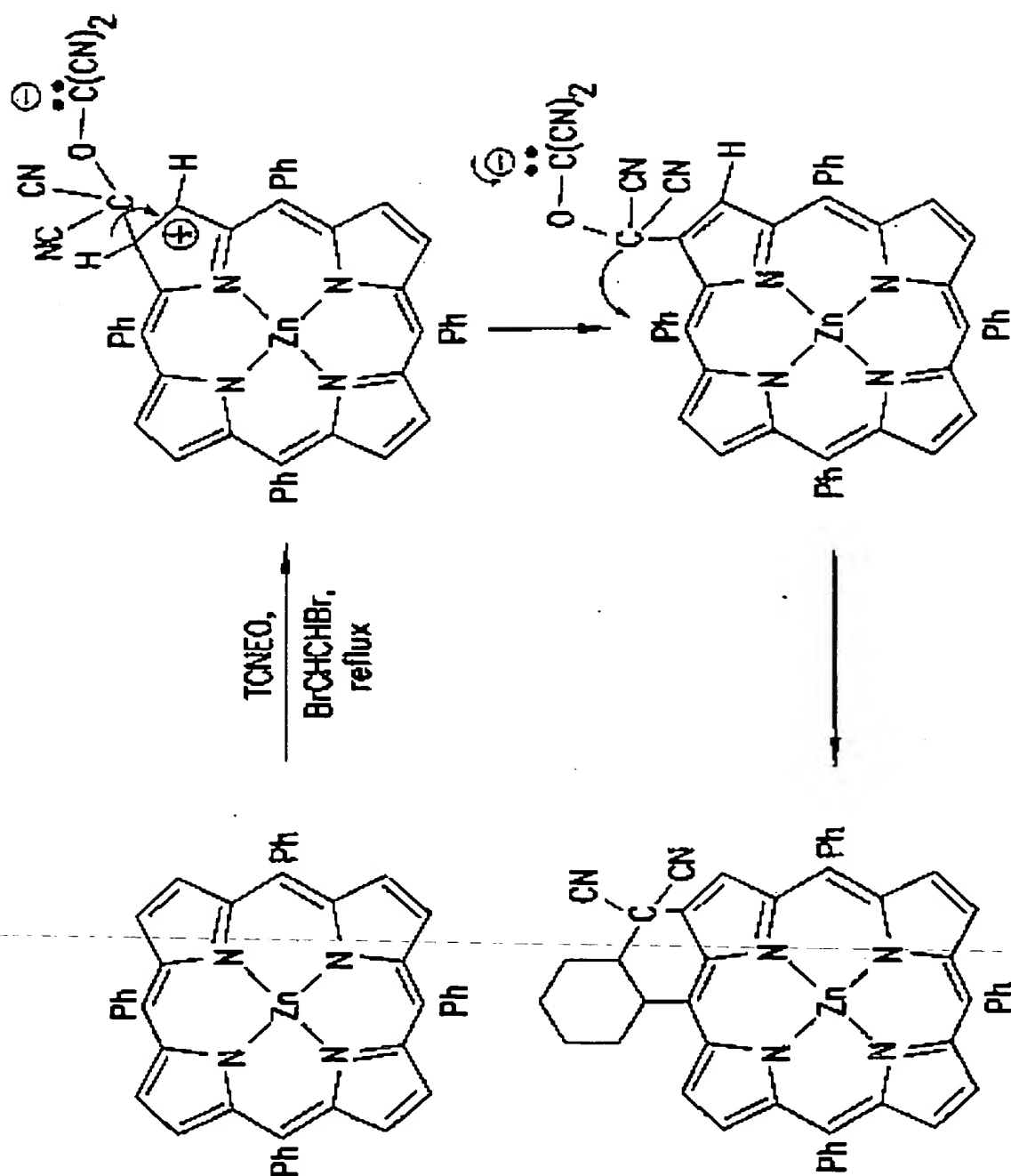


FIG. 8

(218)

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